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Development of Protein-Energy Dense Bar as Malnutrition Ameliorative Strategy Among School Children in Pakistan

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Malnutrition is one amongst the major causes of mortality in children under eight years around the globe. Higher rates of mortality associated with childhood malnutrition and human capital productivity losses are frequently reported from developing countries, particularly those from South Asia. Developing and using cost effective, nutrient rich formulations from indigenous food resources may substantially help address this critical nutritional problem of malnutrition. Given this context, this study was executed to develop a protein-energy-rich food bar from locally sourced animal and plant-based ingredients. Bars were evaluated for organoleptic acceptability, microbiological stability and nutritional composition. The protein-energy dense bar demonstrated a substantial supply of energy amounting to 407.04 kcal/100g and excellent microbiological stability. Total plate count decreased from 1.17±0.04 CFU × 10^2/g to undetectable by 180 days, while yeast & mold counts declined from 1.80 ±0.07 CFU × 10^2/g to undetectable by 90 and 180 days, indicating microbial safety of the bar for 6 months under ambient conditions. Sensory evaluation showed that protein-energy dense bars were generally acceptable, with moderate scores for taste, color, and aroma, suggesting potential for enhancing overall sensory quality. A Bioassay study of 28 days was conducted on 50 malnourished school children aged 4-8, to evaluate protein-energy dense bar on anthropometric, serum proteins and hematological parameters. Participants were divided into a control group and four supplemented groups of 5 to 8 years old children, 10 children each. Supplementation in children significantly improved anthropometric measures (weight gain up to 4.98 kg, BMI >15.5 kg/m²) and hematological parameters (RBCs: 4.38 to 4.77×10^{12}/L; HGB: 11.73 g/dL). Serum protein levels increased markedly in 7-year-olds (6.29 to 7.65 g/dL), indicating substantial nutritional benefits. These findings support the effectiveness of protein-energy-rich dietary bars as a strategy to help reduce protein-energy malnutrition in preschoolers.

Keywords: Protein-Energy Malnutrition, Micronutrient Deficiencies, Stunting, Wasting, School Children, South Punjab, Pakistan.

INTRODUCTION

Protein-energy malnutrition and micronutrient deficiencies continue to distress populations worldwide with more severe health consequences in developing countries. Marasmus and kwashiorkor have been shown to elicit increased health risks together with deficiencies in vitamin A & D, iron, zinc, iodine and calcium in resource constrained countries (Akhtar et al., 2013; Muller and Krawinkel, 2005). Malnutrition distresses a huge chunk of the global population in its various forms and the extent of health damage it elicits is enormous. Two tiers of malnutrition, based on level of wasting i.e. severe acute malnutrition (SAM) and moderate acute malnutrition (MAM) have been perilous and correspond to increased global health burden (Lelijveld et al., 2018; Akhtar, 2016). UNICEF, WHO and World Bank demonstrated that 151million children to be stunted worldwide, 51 million wasted and 38 million were overweight. Asian and Saharan countries were reported to endure increased prevalence of stunting (142.3 million) and wasting (48.8 million) among children of the global count

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(UNICEF, 2018).Undernutrition has been a matter of grave concern for its overwhelming health impact in terms of stunting, wasting and micronutrient deficiencies in developing societies (Frongillo et al., 1997). Unattended and untreated acute malnutrition can be fatal (Tull, 2018). A cross-sectional study conducted in Southern Punjab reported that 78.5% of children under five were malnourished. Based on anthropometric assessments, 56.3% had severe acute malnutrition (SAM) and 25.2% had moderate acute malnutrition (MAM) as per MUAC measurements (Ali et al., 2020). Estimates indicate that undernutrition contributes to approximately 3.1 million deaths among children under 5 each year (Bhutta et al., 2013).

Food-based strategies to avert micronutrient malnutrition have not been substantially focused on the developing world. Available diets are not nutrient dense and therefore fail to supply essential amounts of energy and micronutrients, resulting in nutritional disorders. Consequently, insufficient intake of nutritionally balanced food brings about physiological dysfunction, leading to poor growth and development (Tontisirin et al., 2002). Akram et al., (2023) emphasized the role of nutrition-oriented product development in addressing dietary inadequacies and supporting child health outcomes in South Asian context. Another group of researchers evaluated biscuits made with defatted soy flour and bread wheat flour for their nutrition profile suggesting biscuits to be substantially rich in protein to prevent protein energy malnutrition in children aged 3-8 years (Serrem et al., 2011). Similarly, traditional Indian sweets were made by using partially defatted peanut flour and dehydrated spinach leaves powder as a viable approach to prevent malnutrition among children (Dhanesh et al., 2018).

Supplementary feeding interventions with ready to use therapeutic foods (RUTF) are known to provide effective coverage against moderate acute malnutrition in community-based settings. The term RUTF is coined for food presented in either compressed or spread forms to treat severe malnourishment (Manary, 2006). RUTF are microbiologically stable, energy and nutrient dense pastes that can anticipate appreciable recovery rates against malnourishment (Ciliberto et al., 2005; Ndekha et al., 2005; Manary, 2004; Sandige et al., 2004; Briend, 2001). Dry and solid RUTF remains an effective intervention for malnutrition (Diop et al., 2003). RUTF are preferably derived from the local food crops by using basic production technology and are purposely served uncooked to avoid nutrient losses (Collins and Henry, 2004; Sandige et al., 2004). Clinical trials on malnourished children have suggested soya based RUTF to dispense better weight gain, early recovery and discharge rates than standard inpatient treatment formulas like F100 (Ciliberto et al., 2005; Manary et al., 2004; Diop et al., 2003). F-75 and F-100 have been regarded as potential means for treating malnutrition as proposed by the World health organization in Pakistan. RUTF formulas containing a blend of powdered milk, sugar, and other ingredients such as carbohydrates and essential micronutrients provide an energy value of 520-550 kcal/100g (WHO, 1999). Home based RUTF formulas in the form of thick pastes, bars and biscuits appeared to be safe with the potential to treat uncomplicated cases of severe acute malnutrition (Gera, 2010).

Food and Agriculture Organization (FAO) and Integrated Child Development Scheme (ICDS) suggest cereals and legumes based supplementary feeding to be served as viable strategy in alleviating risks of malnutrition among children from low socio-economic groups (Natarajan et al., 1993; Malleshi and Desikachar, 1982). Protein energy rich ready to use foods including biscuits and energy dense food bars, carrying cereals and legumes derived from complex carbohydrates, protein, soluble and insoluble fibers, essential lipids, vitamins and minerals are widely acceptable and are available in the market with calories ranging from 170 to 270 kcal. A high protein bar had provided 288kcal/100g, containing 34.1g protein, 23g carbohydrate and 6g fat (Jovanov et al., 2021). Some high protein bars claim to provide 64% of the Daily Value which contains carbohydrates from 21 to 53 grams, fats from 3 to 11 grams, proteins from 4 to 17 grams (Larson et al., 2017). Another group of investigators also developed nutritionally rich cereal bars for physically active people and athletes. Compositional analysis of these bars turned out to be 31 g carbohydrates, 6 g protein, 10 g of fat, and 2.5 grams of fiber with a caloric value of 231 kcal (Grden et al., 2008).

The consequences of malnutrition are devastating, leading to increased economic losses. Global prevalence rates of stunting and wasting in children have been worsening day by day in most of the developing countries, therefore taking appropriate measures by local communities seems to be imperative at all levels to eradicate malnutrition burden. This study aimed to formulate and develop a protein-and-energy dense bar as an effective nutritional intervention to address malnutrition among school children in Pakistan.

MATERIALS AND METHODS

Procuring of the Ingredients: Soybean, raisins and almonds were procured from the local market in Multan, Pakistan. Skimmed milk powder, white oats by Quaker, sugar, unsalted butter and golden syrup were purchased from the nearest shopping mall. All ingredients were procured from reputable suppliers. Purchased ingredients were well-packed and were visually inspected for purity before application. Dry ingredients were stored in airtight containers to ensure quality and reliable proximate composition.

Baking of Bars: Soybeans were washed using tap water followed by air drying in shade, making sure an appropriate dust and dirt control environment was in place. Grinding of the washed soybeans was carried out at a local flour mill to procure fine powdered flour. The flour was stored in airtight

plastic jars in a cool dry place until it was used in protein-energy dense bar preparation. Soy flour and white oats were roasted with constant stirring at 100ºC for 20 minutes (Agume et al., 2017) and 140ºC for 20 minutes duration (Schlormann et al., 2020), respectively in an aluminum pan to get roasted aroma while preserving nutrient content. The mixture was cooled at room temperature and kept aside. Almonds and raisins were washed, air dried and chopped manually. Golden syrup and sugar were heated with a measured amount of water until boiling and caramelization of the sugar was initiated by heating the syrup.

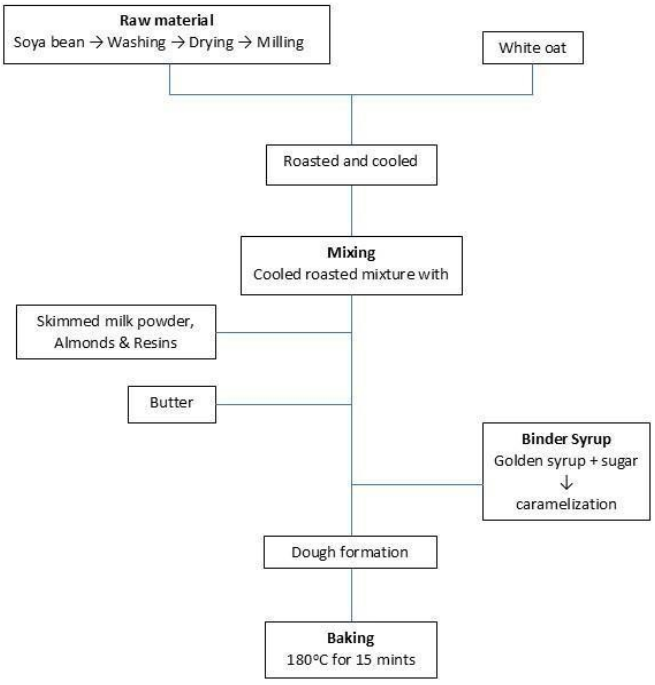

Figure 1. Preparation of Protein-Energy Dense Bar.

Skimmed milk powder, almonds and raisins were thoroughly mixed in the cool roasted mixture. Butter was crumbled into the mixture after thorough mixing of the dry ingredients. Composite dough was formed by adding mixture to the hot binder syrup. Custom built rectangular molds having a capacity of 100 grams were filled with prepared dough and baked at 180ºC for 15 minutes (Figure 1). The custom-built mold for shaping the protein-energy bars was tested using multiple trial batches to ensure uniform size and weight. Bar weight showed minimal variation within acceptable limits, ensuring consistent nutrient distribution as well as sensory and proximate analysis reliability. The packaging of baked products was carried out in aluminum coated polyethylene wrappers and were stored under ambient conditions with temperatures ranging 25ºC to 30ºC and relative humidity of 65% for a period of 180 days in a cool dry place and away from direct sunlight. All ingredients handling was carried under hygienic conditions; dry ingredients were stored in airtight containers to minimize microbial contamination. Protein-energy dense bar preparation was performed using sterilized equipment and utensils. Finished bars were packed in sterilized material and stored at ambient temperature. Microbiological testing confirmed absence of contamination.

Table 1. Recipe of Protein-Energy Dense Bar.

Ingredients	Quantity
Soy flour	20 grams
White oats	20 grams
Skimmed milk powder	15 grams
Almonds	10 grams
Raisins	4 to 5 grains
Butter	10 grams
Corn syrup	15 ml
Water	5 ml
White sugar	5 grams
Vanilla essence	1 drop

Determination of Proximate Composition of Protein-Energy Dense Bars: Proximate composition, i.e. moisture, protein, fats, ash, crude fiber and nitrogen free content of the protein-energy-bar was carried out in triplicates according to the methods described by (AOAC, 2000).

Microbial Analysis of Protein-Energy Dense: Total plate count (TPC) method and spread plate method for yeast and mold estimation were used to determine microbiological status of the bar. 1 g sample was homogenized in 10mL sterile distilled water using Stomacher® 400 Circulator. From this initial mixture 1 mL aliquot was serially diluted to 10^{-6} using 9 mL volume of saline solution. . 1 mL of serially diluted sample was spread onto the nutrient agar and potato dextrose agar (PDA) plates using glass spreaders for TPC and yeast and mold count respectively. The inoculated nutrient agar plates were incubated at 37°C for 24 h while PDA plates were placed in incubator at 25 °C for 3 days. (Stathas et al., 2024). (Yousef et al., 2024). Developed colonies were enumerated with colony counter (Infitek Co, Ltd. Model CC-J2 Shandong, China) and results were expressed as CFU/g. A perfectly stored packed sample was taken out of storage after every 30 days to carry out microbiological safety study. Colony forming units per gram were recorded and used for further analysis on day 0, 30, 60, 90 and 180 of the storage.

Sensory Analysis of Protein-Energy Dense Bars: Sensory evaluation of protein-energy dense bars was performed with 20 trained panelists and school children, who were briefed on the study objectives. Trained panelists underwent a short orientation on the 9-point hedonic scale Aigster et al., (2011) and definitions of sensory attributes (color, taste, texture, aroma, appearance, overall acceptability). Evaluations were conducted under controlled conditions such as consistent serving conditions, uniform lighting, minimal odors and replicate assessments confirmed panel reproducibility. Mean scores were used for analysis.

Performance Assessment: Bioassay study of 28 days was planned to check the effectiveness of protein-energy dense bar on anthropometric and hematological parameters recruiting 50 malnourished schoolers aged 4-8 years. Subjects were screened for chronic illness, birth prematurity, severe and complicated cases of malnutrition to get optimum efficacy of supplementing nutrients through protein-energy dense bars. Subjects were divided into groups (control group receiving only routine diet and intervention groups receiving protein-energy dense bar of 100 grams each day for 28 days). Children supplied with supplemented bars were further divided into 4 subgroups according to their age i.e. 5, 6, 7 and 8 years, each group comprising 10 subjects. A questionnaire regarding personal information, medical history and dietary history were filled by parents or care givers of the subjects under supervision on day 0. Anthropometric indices like weight, height, mid-upper arm circumference and calf circumference were measured at day 0 and day 28 following subjective global nutritional assessment for children, 2007 (Secker and Jeejeebhoy, 2007). Complete blood count and serum analysis for total protein and albumins was conducted at 0 and 28th day of supplementation.

Statistical Analysis: Two factorial design ANOVA between days and groups with treatment variables of all parameters were calculated through software (Statistix 8.1). All comparisons of means regarding anthropometric, hematological and serum chemistry parameters at both days of supplementation were made to determine the effect brought out by supplementation using fisher's LSD test. Prior to performing two factorial design ANOVA, normality of residuals and homogeneity of variances were assessed. Model adequacy was verified through analysis of residuals. All calculations were validated using Statististix 8.1, ensuring reliability of the reported findings for hematological, serum chemistry and anthropometric parameters.

RESULTS AND DISCUSSIONS

Nutritional Profiling: Data presented in Table-2 shows a promising nutritional composition of tested protein-energy dense bar weighing 100 g, demonstrating a substantial supply of energy amounting to 407.04 kcal. Presence of protein and fat in the recipe turned out to be providing 64 and 138.6 kcal respectively. Ash contents indicated in Table-2 are suggestive of increased mineral composition of the formulation while availability of 1.09 g fiber depicts the palatability and ease in digesting these protein-energy bars. Food bars, owing to their natural and healthy composition, have been common in various cultures to override various nutritional deficiencies. However, a paradigm shift had been witnessed from their conventional use to a large-scale exploitation for addressing malnutrition in times of calamities and natural disasters especially in developing regions with increased frequency of natural catastrophes. Several studies have illustrated the potential use of protein-energy dense bars or similar foods for schoolers. One study substantiated the composition of food bar satisfying 84.6% of daily protein needs of children 4-8 years old and with a protein yield of 16g per 100 grams. Likewise, Santos et al., (2011) reported protein content of cereal bars ranging between 4.60% and 4.80%. Protein-energy dense bars were prepared with ingredients such as soy flour, white oats, skimmed milk powder, almonds, raisins, butter, white sugar and corn syrup which are nutritionally ranked as valuable sources of micronutrients. Fiber content of food bars generally comes from fruit, nuts and whole cereals and protein energy bars prepared in the present study showed higher crude fiber contents (1.09%). Lima et al., (2010) have confirmed increased fiber contents (16.73%) in various types of bars such as those made from almond and pulp. Total carbohydrate contents of protein-energy dense bar (Table-2) i.e. 51.11% were lower than cereal-based fruit peel bar(61.61%) made by Mendes et al., (2013).

Table 2. Proximate Composition of Protein Energy Food Bar.

Nutrients	Per 100 grams
Energy	407.04 kcal
Proteins	16g
Fats	15.4g
Moisture	14g
Fiber content	1.09g
Nitrogen free extract	51.11
Ash content	2.4g

Microbiological Evaluation of Protein-Energy Dense Bars: Data pertaining to microbiological status of the protein-energy dense bars during storage for 180 days showed a significant increase in microbial load in the product after the first month of storage at ambient temperature and the decline in total plate count and yeast & molds which continued decreasing after two months i.e. 1.17±0.04 CFU × 10^2.g^{-1} at day 0 which initially increased to 1.12±0.06 at day 30 followed by a significant decrease ($p < 0.05$) to 0.99±0.03 at day 60 and 0.68±0.02 at day 90 (Table-3). However, no colonies for total plate count and yeast & molds could be detected in the product during microbiological analysis at the termination of the storage period (Table-3). Overall, the product remained microbiologically stable for three months showing no significant increase in bacterial and fungal load suggesting sound microbiological safety of protein-energy dense bars. Data depicted total plate count and yeast & molds (CFU × 10^2.g^{-1}) to the tune of 1.80±0.07 unit at day 0 followed by 1.65±0.03 at day 30, 0.55±0.05 at day 60 and were not detected at day 90 and day 180 suggesting that protein energy food bar had a safe shelf life of up-to 6 month if prepared following hygienic food practices. Reduced moisture contents of the product during the storage period primarily render a substantial effect on microbial proliferation leading to good

shelf life (Beuchat, 1981). Another group of researchers confirmed Protein rich composite cereal bars to exhibit good microbiological stability and extended shelf life (up to 3 months) during storage under ambient (15-34°C) conditions (Padmashree et al., 2012). Our results are validated by Azeredo et al., (2004) who supported the notion that increased moisture content favored the growth of bacteria, yeasts, and molds in food bars.

Table 3. Microbial Count Expressed as Colony Forming Units (CFU)/g of Protein Energy Food Bars Stored Under Ambient Conditions (Average of Three Replicates).

	Count on (CFU × 10^2)/g	
Days	**Total Plate Count**	**Yeast and Molds**
0	1.17 ± 0.04^{c}	1.80 ± 0.07^{c}
30	1.12 ± 0.06^{c}	1.65 ± 0.03^{bc}
60	0.99 ± 0.03^{b}	0.55 ± 0.05^{b}
90	0.68 ± 0.02^{a}	Not Detected
180	Not Detected	Not Detected

Sensory Evaluation: Results for sensory evaluation of the protein- energy dense bars have been shown in Figure 2. Bars under sensory evaluation, scoring 5 points or above for all parameters, were rated as acceptable. The product was moderately liked by the panelists for taste and color. However, the color and aroma of the product did not score much higher during sensory evaluation. Addition of soy flour and white oats might have elicited a little deteriorating effect on taste and aroma as these sensory attributes are instrumental for overall acceptability of the product by the consumers (Figure 2). Likewise, assimilation of skim milk powder, butter, white sugar and vanilla flavor obviously rendered a pleasing sensation contributing to enhanced taste and aroma of the product and improvement in the mouthfeel with little residue formation in the mouth. In the same manner, relatively increased water content in the recipe and incorporation of butter improved gumminess and chewiness of the product. Addition of resins and almonds seemingly impacted the color, giving a brownish appetizing tinge to the baked protein-energy dense bars. The overall score for all attributes nevertheless did not exceed 7.0 denoting the room for improvement in the sensory profile of the product (Figure 2). Scientific literature is not scant on sensory profiling of food bars as Freitas and Moretti, (2006) demonstrated significant changes in appearance, color, and overall acceptance during storage of food bars due to packaging types such as darkening of the product due to oxidative reaction by residual oxygen in the pack. Similarly, Aramouni and Abu-Ghoush, (2011) illustrated the role of water activity ($_{aw}$)level of the product as a visible marker of the quality of the food bars. The researchers demonstrated that the food bars with a level below 0.7 could be preserved for about 6 months. The findings further confirmed that color, crust surface, crumb color, texture and mouth feel of the product remained unchanged during storage up to 3 months, our results are in consonance with those of Rawat and Darappa, (2015) who reported good sensorial characteristics of the food bars during storage up to 3 months.

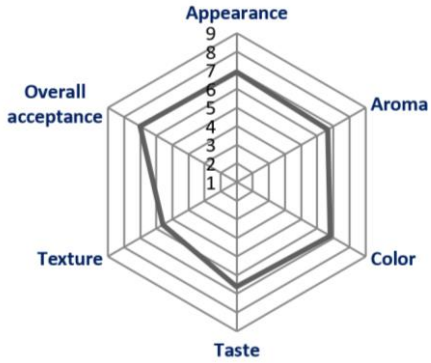

Figure 2. Sensory evaluation of protein-energy dense bars.

Effect on Anthropometric Measurements: ANOVA results on anthropometrics measurements showed a highly significant increment in weight, BMI, MUAC and calf circumference (Table 4). Mean comparison of weight at day 0 with day 28 for the whole sample indicated weight gain of 2.8 kg; these readings concede weight gain of 3.1 kg as reported by Norman et al., (2008) after oral supplementation of three months. The highest value of weight increment was recorded in supplemented groups of 7 years old with an average value of 4.98 kg weight. Weight for age percentile shifted from initial values of 42.820±25.24 (mean±SD) to final values of 66.67±26.35 (mean±SD). Patel et al., (2005) achieved weight for age percentile up-to mean value 66±9 with supplementary feedings of corn soy blend and ready-to-use food. Supplementation did not bring out any significant effect on height in the subjects. Supplementation of 28 days helped the subjects to pass the threshold of severely and moderately malnourishment to mild malnutrition conditions as body mass index at the end of supplementation for all the subjects was recorded above 15.5kg/m^2.

MUAC and calf circumference were the most important parameters in case of protein energy malnutrition and both parameters were significantly increased after the supplementation. Supplementation provided by Patel et al., (2005) affected MUAC with mean increment of 0.5 cm, which is less than mean increment value of 0.7 cm in MUAC obtained in this study. An increase of 0.925 cm on average was recorded in calf circumference at day 28.

Table 4. Effect of Supplementation on Anthropometric Measurements.

Treatment groups	Days	Weight	Weight for age	Height	Height for age	BMI	MUAC	Calf circumference
Control	0	23.06±2.21^{a}	31.50±20.20^{de}	52.00±2.20^{a}	66.70±24.75^{b}	13.62±1.18^{b}	15.88±0.76^{b}	21.52±1.31^{a}
	28	23.26±1.58^{a}	29.50±18.70^{e}	52.25±2.23^{a}	66.70±24.75^{b}	13.34±1.30^{b}	15.90±0.66^{b}	21.77±1.25^{a}
Supplemented	0	15.60±1.90^{e}	36.00±23.70^{cde}	42.10±1.94^{f}	65.60±33.10^{b}	14.10±1.70^{b}	14.25±0.63^{c}	18.70±1.23^{d}
(5 years)	28	18.32±1.88^{cd}	73.20±20.26^{a}	42.60±1.84^{f}	66.10±33.60^{b}	15.67±1.49^{a}	14.66±0.60^{c}	19.49±1.06^{cd}
Supplemented	0	18.07±2.87^{c}	47.30±29.17^{bc}	44.97±2.45^{e}	66.50±36.05^{b}	14.17±1.71^{b}	14.65±1.02^{c}	19.39±1.65^{cd}
(6 years)	28	20.95±2.74^{b}	73.50±23.51^{a}	45.50±2.24^{de}	71.00±30.15^{ab}	15.76±2.26^{a}	15.66±0.84^{b}	19.91±2.40^{c}
Supplemented	0	19.06±4.87^{cd}	43.80±23.60^{bcd}	48.74±3.73^{b}	78.00±16.09^{ab}	13.59±1.51^{b}	15.55±1.13^{b}	19.57±1.50^{cd}
(7 years)	28	24.04±3.74^{a}	74.15±18.60^{a}	49.15±3.68^{b}	78.00±16.09^{ab}	15.64±1.58^{a}	16.79±1.33^{a}	21.45±1.16^{a}
Supplemented	0	19.84±2.31^{bc}	55.50±24.00^{b}	46.90±1.89^{cd}	80.00±19.70^{ab}	14.28±1.26^{b}	14.71±1.03^{c}	20.16±1.84^{bc}
(8 years)	28	23.03±1.87^{a}	83.00±8.33^{a}	47.10±1.71^{c}	83.50±12.36^{a}	16.11±1.05^{a}	15.56±0.96^{b}	21.09±1.80^{ab}

Mean ± standard deviation. Data of a specific product in the same column bearing similar lettering do not differ significantly at $p \leq 0.05$.

Effect on Hematological Parameters: After investigating hematology reports at day 0 and day 28, it is safe to say that protein energy food bar supplementation didn't impose any drastic effect on subjects hematological parameters, it did not cause any negative effect on the blood cell levels except that it increased red blood cells concentration towards the normal ranges and helped to decreases raised white blood cell levels to the normal level's ranges.

Table 5 shows the effect of supplementation on the hematological profile of school-aged children over 28 days. A marked improvement in red blood cell count (RBCs) was observed among all supplemented groups, with the most notable increase in the 8-year-old group (4.38 to 4.77×10¹²/L), indicating enhanced hematopoiesis likely due to improved nutritional intake. Hemoglobin levels (HGB) also increased across all supplemented age groups, with values reaching up to 11.73 g/dL, suggesting a positive impact on iron status. Hematocrit (HCT) values showed a corresponding rise, particularly among 6- and 7-year-olds, reflecting better red cell mass and oxygen-carrying capacity. The mean corpuscular volume (MCV) remained relatively stable, falling within the normal range, which may imply balanced erythropoiesis without macrocytic or microcytic shifts. Platelet counts improved in most supplemented groups, with the highest post-supplementation level seen in 6-year-olds (368.5×10⁹/L), further indicating the hematological benefits of the intervention. A general decline in white blood cell counts (WBCs) was observed in all groups, including controls, though values remained within physiological limits, possibly reflecting reduced inflammation or stress. Lymphocyte percentages also remained within the normal range and showed mild fluctuations post-supplementation. Similar trends were observed by Dewey and Adu-Afarwuah, (2008), who documented enhanced red cell indices and reduced anemia in children receiving lipid-based nutritional supplements. Yakoob and Bhutta, (2011) also emphasized the positive hematological impact of food-based interventions in malnourished populations of low-income countries. Collectively, these findings suggest that short-term supplementation with a protein-energy dense bar can beneficially influence hematological status in growing children.

Effect on Serum Chemistry: The effect of supplementation on serum chemistry parameters, specifically total proteins and albumin levels, is presented in Table 6. Among the supplemented groups, a notable increase in total protein levels was observed in the 7-year-old children, rising from 6.29 to 7.65 g/dL over 28 days, which was significantly higher than other groups and the control. This suggests a positive response to protein-energy supplementation in this age group. Albumin levels also improved significantly in the same group, increasing from 5.26 to 6.14 g/dL, indicating enhanced

Table 5. Effect of Supplementation on Subject's Hematology.

Treatment groups	Days	WBCs	RBCs	Lymph %	HGB	HCT	MCV	PLTs
Control	0	12.73±2.04^{a}	3.60±0.82^{e}	36.92±5.94^{b}	10.57±2.36^{b}	27.17±5.75^{d}	76.54±9.09^{a}	295.95±90.45^{cd}
	28	9.78±1.93^{b}	4.27±0.39^{bcd}	40.76±7.27^{ab}	11.28±1.23^{ab}	30.74±3.55^{abcd}	72.08±8.67^{ab}	281.35±56.45^{d}
Supplemented	0	12.77±2.86^{a}	4.02±0.69^{de}	41.35±8.07^{ab}	11.24±0.60^{ab}	29.60±1.32^{bc}	75.53±9.24^{ab}	297.70±74.30^{cd}
(5 years)	28	9.49±1.61^{b}	4.35±0.65^{a-d}	42.34±5.69^{a}	11.03±0.82^{ab}	29.66±1.96^{bc}	72.07±7.57^{ab}	281.30±68.01^{d}
Supplemented	0	12.46±2.33^{a}	4.04±0.56^{cde}	41.31±7.21^{ab}	11.26±0.99^{ab}	29.12±2.23^{cd}	73.50±5.57^{ab}	317.1 ±71.6^{bcd}
(6 years)	28	9.20±2.18^{b}	4.47±0.41^{abc}	42.33±7.68^{a}	11.53±1.57^{a}	31.73±3.33^{ab}	71.76±5.79^{b}	368.50±113.43^{a}
Supplemented	0	12.52±2.30^{a}	4.04±0.43^{cd}	42.43±6.00^{a}	11.68±1.14^{a}	31.05±2.61^{abc}	75.82±8.85^{ab}	351.75±74.52^{ab}
(7 years)	28	9.43±1.50^{b}	4.51±0.32^{ab}	41.30±5.42^{ab}	11.73±0.93^{a}	32.26±1.76^{a}	74.34±6.50^{ab}	335.8±95.76^{abc}
Supplemented	0	12.78±2.36^{a}	4.38±0.96^{a-d}	37.62±7.33^{b}	11.39±1.18^{ab}	32.19±5.76^{a}	74.81±5.17^{ab}	276.95±65.57^{d}
(8 years)	28	8.95±2.05^{b}	4.77±1.22^{a}	40.45±9.47^{ab}	11.62±1.77^{a}	31.08±5.33^{abc}	73.11±4.61^{ab}	308.3±88.83^{bcd}

Mean ± standard deviation. Data of a specific product in the same column bearing similar lettering do not differ significantly at $p \leq 0.05$.

protein synthesis and improved nutritional status. In contrast, other supplemented groups did not show statistically significant increases; in fact, mild declines in albumin were observed in the 5- and 6-year-old groups, which may be attributed to individual variability or differences in baseline nutritional status. The control group showed a slight decrease in both serum total proteins and albumin levels over the study period, suggesting the absence of nutritional support may have contributed to the decline.

The marked improvements observed in the 7-year-old group align with findings from studies such as Ahmed et al., (2013), where nutritional interventions led to increased serum protein levels in malnourished children. Similarly, Ghosh et al., (2013) reported significant gains in serum albumin following a protein-rich dietary intervention in undernourished school-aged children. Improvements in serum protein levels serve as a biochemical indicator of enhanced nutritional intake and metabolic recovery. These results underscore the potential of age-targeted supplementation in improving serum chemistry markers and overall nutritional status among school-going children.

Table 6. Effect of Supplementation on Serum Chemistry.

Treatment Groups	Days	Total protein	Albumin
Control	0	6.37 ± 1.15^{BC}	5.68 ± 0.5^{B}
	28	6.34 ± 0.75^{BC}	5.49 ± 0.4^{BC}
Supplemented	0	6.85 ± 1.1^{B}	5.35 ± 0.6^{BC}
(5 years)	28	6.45 ± 1.0^{BC}	5.27 ± 0.8^{CD}
Supplemented	0	6.50 ± 0.8^{BC}	5.44 ± 0.4^{BC}
(6 years)	28	6.82 ± 1.3^{BC}	4.92 ± 0.5^{D}
Supplemented	0	6.29 ± 1.2^{BC}	5.26 ± 0.7^{CD}
(7 years)	28	7.65 ± 1.3^{A}	6.14 ± 0.3^{A}
Supplemented	0	6.09 ± 1.0^{C}	5.50 ± 0.4^{BC}
(8 years)	28	6.81 ± 1.5^{BC}	5.31 ± 0.4^{C}

Mean ± standard deviation. Data of a specific product in the same column bearing similar lettering does not differ significantly at $p \leq 0.05$.

***Conclusion*:** The study validates the protein-energy dense bars as a safe, shelf-stable, and nutritionally effective intervention for combating protein-energy malnutrition. Lower moisture content of the bar supported microbiological stability without a significant decline in sensory attributes, however; the study indicated room for refinement of the sensory profiles by further optimizing ingredients e.g. soy flour. Protein-energy bar supplementation significantly enhanced weight, BMI, MUAC and hematological markers in children, with pronounced improvements in serum proteins among 7-year-olds. These findings align with global evidence on food-bar fortification, advocating for scalable deployment in resource-limited settings. Future work on locally developed protein-energy bars should optimize sensory attributes of the product and explore its long-term physiological impacts in preschoolers.

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***Conflict of interest*:** The authors confirm that there are no known financial or personal conflicts that could have reasonably been perceived as influencing research, reported in this paper.

***Ethical statement*:** The study was conducted in accordance with established ethical guidelines and institutional regulations and received approval from the relevant ethics review committee before data collection.

***Availability of data*:** Data supporting the findings of this study are available within this article.

***Consent to participate*:** All participants/legal guardians voluntarily consented to participate in the study after being informed about the objectives and procedures.

***Informed consent*:** Participants involved in this research were under 18, informed consent was obtained from their parents.

***Consent for publication*:** All authors consented to the publication for this manuscript. Participants whose information is presented in this study also provided consent for its publication.

***SDGs addressed*:** This study supports United Nations Sustainable Development Goals, specifically SGD 2 (Zero Hunger), SDG 3 (Good Health and Well-Being) and SDG 4 (Quality Education). Protein-energy bar supplementation has improved nutritional status, health markers and cognitive development/school performance; aiding in combating malnutrition in school-age children.

***Policy referred*:** The study aligns with national and international nutrition policies and guidelines including the World Health Organization (WHO) recommendations for prevention and management of severe and moderate acute malnutrition in children, and the Pakistan National Nutrition Program framework aimed at reducing childhood stunting and wasting.

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